

**Global Mitotic Phosphorylation of C<sub>2</sub>H<sub>2</sub> Zinc Finger Protein  
Linker Peptides**

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Supplementary Data

Supplementary Table 1 and Supplementary Figures 1-6

Supplementary Table 1 Examples of TGEKP and/or pTGEKP-containing zinc finger proteins.

Protein name	AA	TGEKP Seq. position	Accession
Aiolos	509	169-173 <sup>1</sup>	NP_036613
BCL-11A	835	400-405	NP_075044
CTCF	727	518-522	NP_006556
EGR1	543	391-395	NP_001955
FLT3-interacting zinc finger 1	496	102-106; 437-441	NP_116225
GFI1	422	363-367	NP_001120688
GLI1	1106	296-300; 326-330	NP_005260
HKR1	659	380-384;436-440;492-496	NP_861451
IKZF5	419	105-109; 133-137	NP_071911
KLF1	362	304-308	NP_006554
MTF1	753	254-258	NP_005946
Myoneurin	610	353-357;438-442;466-470	NP_001172047
Myeloid zinc finger 1	734	592-596; 620-624	NP_003413
RBAK	714	284-288;424-428;590-594	NP_001191385
SALL1	1324	1157-1161	NP_002959
SCRT2	307	235-239	NP_149120
SNAI1	264	203-207	NP_005976
Transcription Factor IIIA	365	95-99	NP_002088
TIP20	382	117-121 <sup>1, 2</sup> ; 145-149 <sup>2</sup>	NP_001002836
WT1	497	399-403;457-461	NP_000369
YY1	414	348-352 <sup>1, 3-5</sup>	NP_003394
YY2	372	306-310 <sup>1, 4, 5</sup>	NP_996806
ZBTB7B	539	369-373 <sup>1</sup>	NP_056956
ZBTB16	673	569-573 <sup>6</sup>	NP_001018011
ZNF24	368	302-306 <sup>6</sup> ;330-334 <sup>6</sup>	NP_008896
ZNF28	718	350-354;378-382;406-410 <sup>6</sup> ;434-438;490-494; 518-522;574-578;602-606;630-634;686-690	NP_008900
ZNF100	542	256-260;284-288;340-344;424-428 <sup>6</sup> ; 452-456 <sup>6</sup> ;480-484 <sup>6</sup>	NP_775802

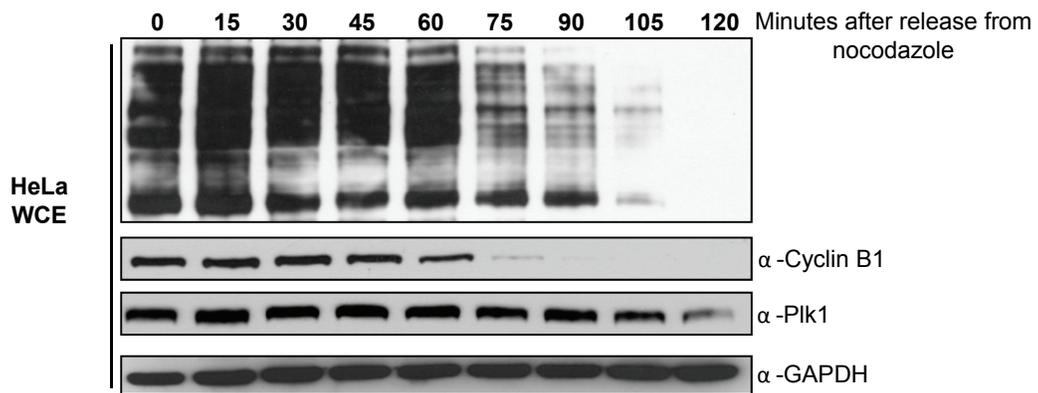
ZNF107	783	<b>155-159</b> <sup>g</sup> ;211-215;351-355;435-439;463-467; <b>491-495</b> <sup>g</sup> ;547-551; <b>631-635</b> <sup>g</sup> ;659-663; 687-691;743-747;771-775	NP_001013768
ZNF117	483	<b>160-164</b> <sup>g</sup> ;328-332;356-360	NP_056936
ZNF148	794	<b>194-198</b> <sup>1</sup> ;222-226	NP_068799
ZNF155	538	227-231;255-259; <b>283-287</b> <sup>1</sup> ;311-315;339-343; 367-371	NP_003436
ZNF187	478	333-337; <b>417-421</b> <sup>1</sup>	NP_001104509
ZNF195	629	<b>433-437</b> <sup>g</sup> ;461-465;517-521; <b>545-549</b> <sup>g</sup> ;573-577; <b>601-605</b> <sup>g</sup>	NP_001123992
ZNF221	617	249-253;277-281; <b>305-309</b> <sup>1</sup> ;333-337;389-393	NP_037491
ZNF225	706	<b>227-231</b> <sup>1</sup> ; <b>283-287</b> <sup>1</sup> ;367-371;619-623	NP_037494
ZNF226	803	<b>386-390</b> <sup>1</sup> ;414-418;442-446;498-502;526-530; 582-586;610-614;694-698;722-726;750-754	NP_001027545
ZNF234	700	<b>283-287</b> <sup>1</sup> ;311-315;339-343;367-371;395-399; 423-427;479-483;507-511;563-567;591-595; 619-623;647-651	NP_006621
ZNF230	474	<b>219-223</b> <sup>1</sup> ;247-251;275-279	NP_006291
ZNF235	738	338-342;366-370;394-398;422-426;478-482; 506-510;534-538;562-566;590-594;618-622; <b>646-650</b> <sup>1</sup> ;674-678	NP_004225
ZNF254	659	345-349;429-433;485-489;513-517; <b>569-573</b> <sup>g</sup>	NP_975011
ZNF267	743	403-407; <b>431-435</b> <sup>g</sup> ;487-491;543-547;571-575; 599-603	NP_003405
ZNF283	679	258-262;286-290;342-346;398-402;426-430; 454-458;510-514;538-542; <b>566-570</b> <sup>1</sup>	NP_862828
ZNF407	2248	<b>1651-1655</b> <sup>1</sup> ;1709-1713;1737-1741	NP_060227
ZNF479	524	264-268;320-324;376-380; <b>488-492</b> <sup>g</sup> ;516-520	NP_150376
ZNF454	522	239-243;267-271; <b>295-299</b> <sup>1</sup> ;351-355;379-383; 407-411;435-439;491-495	NP_872400
ZFP1	407	<b>207-211</b> <sup>g</sup> ; 235-239; 347-351; 375-379	NP_710155
ZFP91	570	<b>395-399</b> <sup>1 g</sup>	NP_444251
ZFP95	839	<b>369-373</b> <sup>1</sup> ; 397-401; 572-576; 600-604; 796-800	NP_659570
ZFX	805	<b>571-575</b> <sup>2</sup>	NP_001171556
ZFY	801	<b>567-571</b> <sup>2</sup>	NP_003402

50 examples of zinc finger proteins, their sizes (in amino acid residues), NCBI accession number, and location(s) of TGEKP amino acid sequence(s). Only TGEKP linker peptides were been considered in this search and displayed in this table. All TGEKP linker peptides of each of the listed proteins have been indicated with their amino acid location(s) within each protein. TGEKP linker peptides that have been reported to be phosphorylated are indicated (red color), with the corresponding reference(s) of their report(s).

References for these reported phosphorylation sites:

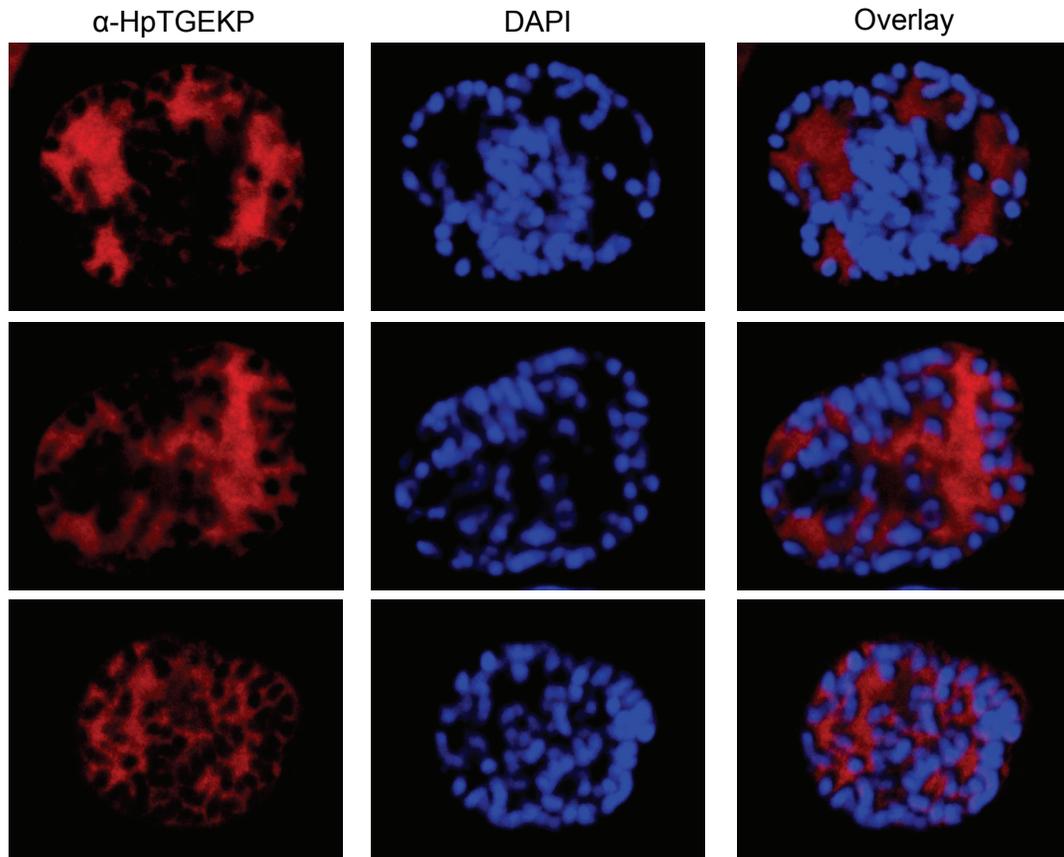
1. Dephoure N, Zhou C, Villen J, Beausoleil SA, Bakalarski CE, Elledge SJ, et al. A quantitative atlas of mitotic phosphorylation. *Proc Natl Acad Sci U S A* 2008; 105:10762-7.
2. Chen RQ, Yang QK, Lu BW, Yi W, Cantin G, Chen YL, et al. CDC25B mediates rapamycin-induced oncogenic responses in cancer cells. *Cancer Res* 2009; 69:2663-8.
3. Rizkallah R, Hurt MM. Regulation of the transcription factor YY1 in mitosis through phosphorylation of its DNA-binding domain. *Mol Biol Cell* 2009; 20:4766-76.
4. Mayya V, Lundgren DH, Hwang SI, Rezaul K, Wu L, Eng JK, et al. Quantitative phosphoproteomic analysis of T cell receptor signaling reveals system-wide modulation of protein-protein interactions. *Sci Signal* 2009; 2:ra46.
5. Olsen JV, Vermeulen M, Santamaria A, Kumar C, Miller ML, Jensen LJ, et al. Quantitative phosphoproteomics reveals widespread full phosphorylation site occupancy during mitosis. *Sci Signal* 2010; 3:ra3.
6. Moritz A, Li Y, Guo A, Villen J, Wang Y, MacNeill J, et al. Akt-RSK-S6 kinase signaling networks activated by oncogenic receptor tyrosine kinases. *Sci Signal* 2010; 3:ra64.

## Supplementary Figure 1



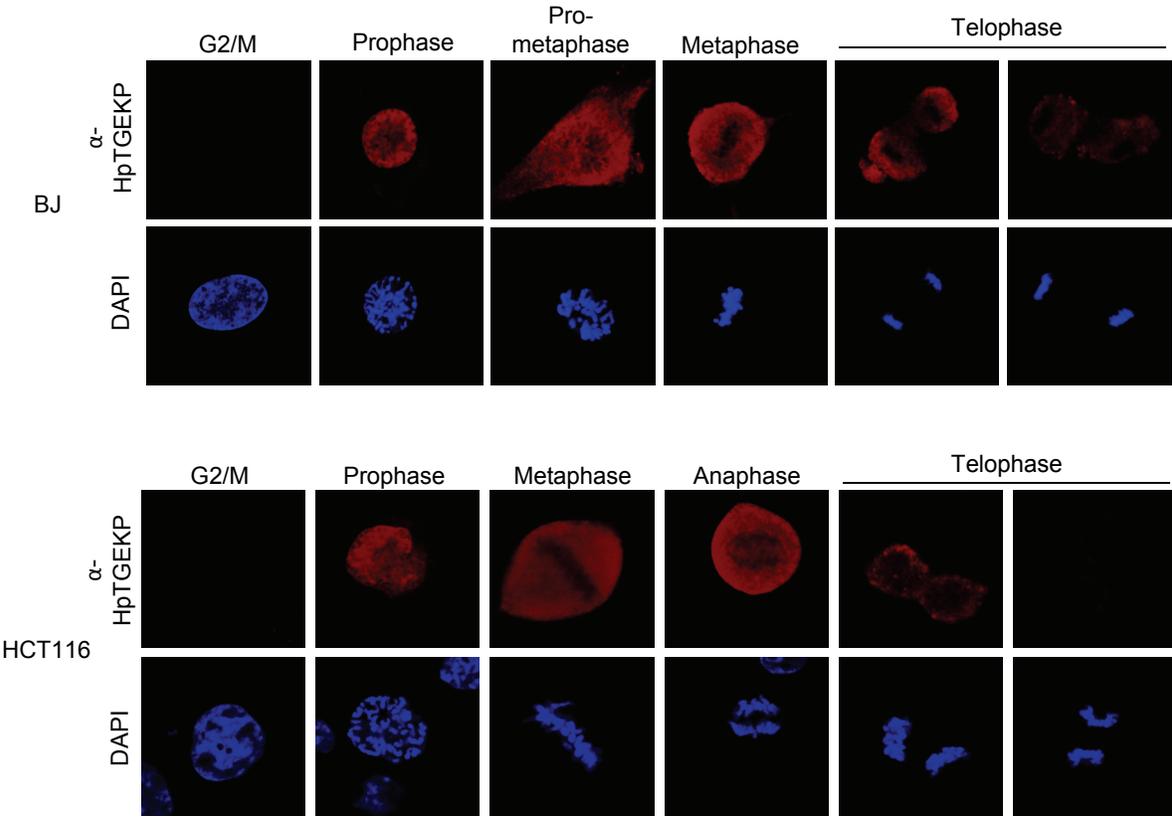
**Supplementary Figure 1.** Western blot of WCEs from HeLa cells synchronized at pro-metaphase with nocodazole and released for the indicated time points. The blot was probed with  $\alpha$ -HpTGEKP, then stripped and reprobbed with  $\alpha$ -GAPDH as a loading control, and  $\alpha$ -cyclin B1 and  $\alpha$ -Plk1 to monitor progression through mitosis.

## Supplementary Figure 2



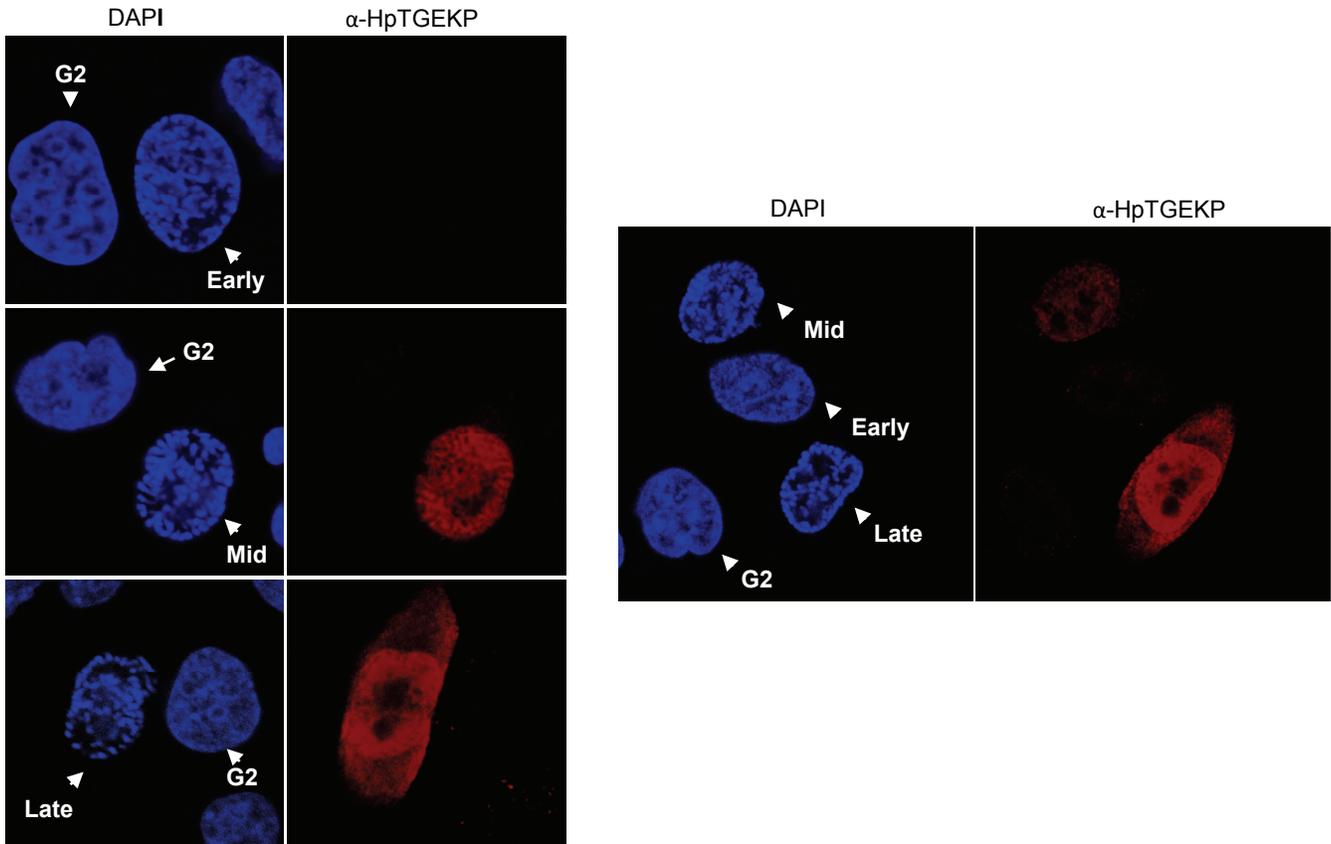
**Supplementary Figure 2.** Exclusion of HpTGEKP signal from condensed DNA in prophase. HeLa cells were grown on coverslips, synchronized with double thymidine and released for 8 hours. Cells were fixed and immunostained with  $\alpha$ -HpTGEKP (red) and DAPI (blue). Images of three HeLa cells in prophase were captured on a fluorescent confocal microscope.

### Supplementary Figure 3



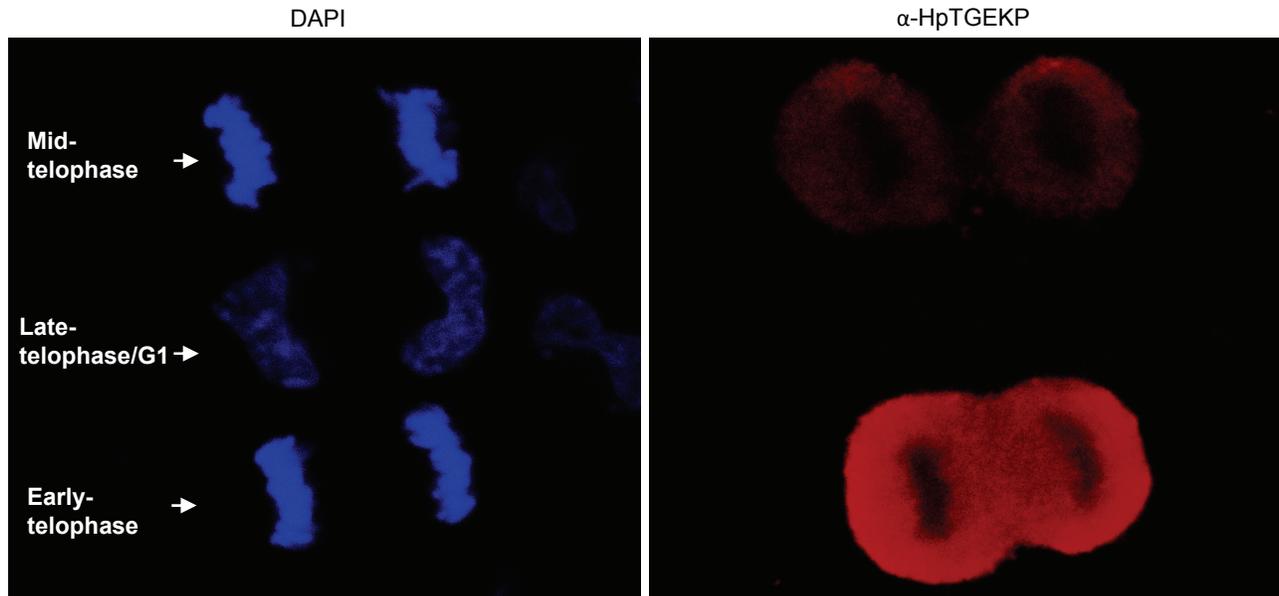
**Supplementary Figure 3.** HpTGEKP timing and distribution in BJ and HCT116 cell lines. BJ and HCT116 were grown on coverslips, synchronized with thymidine and released for 8-11 hours. Cells were then fixed, permeabilized, and immunostained with  $\alpha$ -HpTGEKP (red) and DAPI (blue). Images were captured on a fluorescent confocal microscope. Specific mitotic stage labeling was based on chromatin morphology.

## Supplementary Figure 4



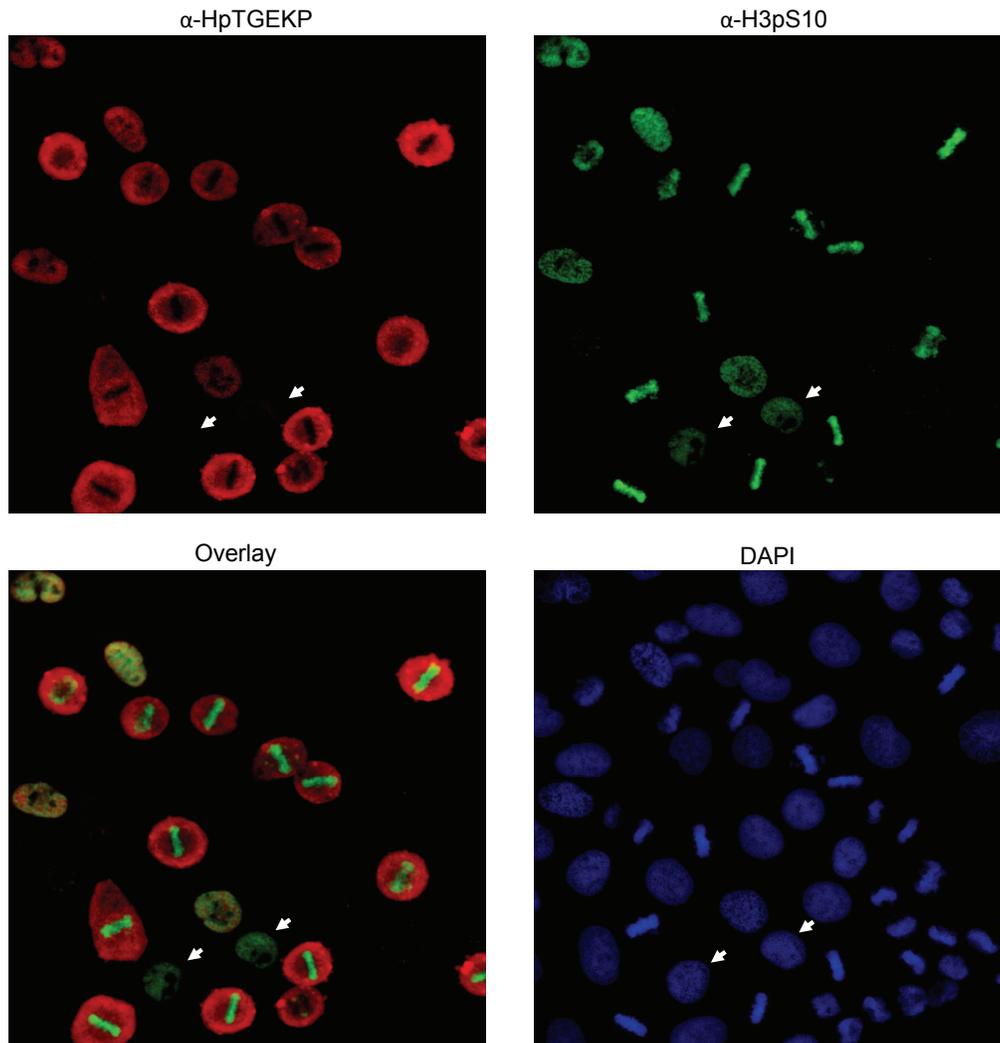
**Supplementary Figure 4.** Timing of HpTGEKP phosphorylation and cellular distribution during prophase. HeLa cells were grown on coverslips synchronized by double-thymidine block and released for 8.5 hours, fixed, permeabilized, and stained with DAPI (blue) and  $\alpha$ -HpTGEKP (red). Images were captured on a fluorescent confocal microscope. Images show cells in late G2, early, mid, and late prophase based on their chromatin morphology.

## Supplementary Figure 5



**Supplementary Figure 5.** HpTGEKP dephosphorylation during telophase. HeLa cells were grown on coverslips synchronized by double-thymidine block and released for 9.5 hours, fixed, permeabilized, and stained with DAPI (blue) and  $\alpha$ -HpTGEKP (red). The image was captured with a fluorescent confocal microscope and shows three cells at the end of mitosis, at three different stages of telophase, based on their chromatin morphology, and the extent of the cleavage furrow of cytokinesis.

## Supplementary Figure 6



**Supplementary Figure 6.** pH3S10 phosphorylation briefly precedes that of HpTGEKP. This confocal image shows a mixed population of HeLa cells at different stages of G2, prophase and metaphase. HeLa cells were grown on coverslips synchronized with a single thymidine block and released for  $\sim 9$  hours, fixed, permeabilized, and stained with DAPI (blue),  $\alpha$ -HpTGEKP (red), and pH3S10 (green). This image shows that most mitotic cells display both  $\alpha$ -HpTGEKP and pH3S10 phosphorylation. However, two cells have pH3S10 phosphorylation prior to HpTGEKP phosphorylation, as indicated by arrows.